APPLICATION UNDER UNITED STATES PATENT LAWS

Invention: NATAMYCIN DOSAGE FORM, METHOD FOR PREPARING SAME AND USE THEREOF

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This is a:

[ ] Provisional Application

[X] Regular Utility Application

[ ] Continuing Prosecution Application

[ ] PCT National Phase Application

[ ] Design Application

[ ] Reissue Application

[ ] Plant Application

Natamycin dosage form, method for preparing same and use thereof

Claim of priority

This application claims priority under 35 U.S.C. § 119 to GB application no. 0319817.3, filed August 22, 2003, the entire contents of which have been incorporated by reference in its entirety.

Field of the invention

The present invention relates to natamycin dosage forms for the food industry, and more particularly to microcapsules where natamycin as the active food preservative ingredient is encapsulated within a shell. The present invention relates also to novel methods for preparing the microcapsules according to the invention, to the use of the microcapsules of the present invention in the food industry, as well as to food products containing the same. Preferred food products include acidic food products and sliced bread.

Background of the invention Natamycin is a polyene macrolide natural antifungal agent produced by fermentation of the bacterium *Streptomyces natalensis.* Natamycin (previously known as pimaricin) has an extremely effective and selective mode of action against a very broad spectrum of common food spoilage yeasts and moulds with most strains being inhibited by concentrations of 1-15 ppm of natamycin.

Natamycin is accepted as a food preservative and used world wide, particularly for surface treatment of cheese and dried fermented sausages. It has several advantages as a food preservative, including broad activity spectrum, efficacy at low concentrations, lack of resistance, and activity over a wide pH range. Neutral aqueous suspensions of natamycin are quite stable, but natamycin has poor stability in acid or alkaline conditions, in the presence of light, oxidants and heavy metals. For example, natamycin can be used in pasteurised fruit juice to prevent spoilage by heat-resistant moulds such as *Byssochlamys.* The acid pH of the juice, however, promotes degradation of natamycin during pasteurisation as well as during storage if the juice is

not refrigerated. Natamycin is also degraded by high temperature heat processing, such as occurs during cooking of bakery items in an oven.

At extreme pH conditions, such as pH less than 4 and greater than 10, natamycin is rapidly inactivated with formation of various kinds of decomposition products. Acid hydrolysis of natamycin liberates the inactive aminosugar mycosamine. Further degradation reactions result in formation of dimers with a triene rather than a tetraene group. Heating at low pH may also result in decarboxylation of the aglycone. Alkaline hydrolysis results in saponification of the lactone. Both acid degradation products (aponatamycin, the aglycone dimer, and mycosamine), and alkaline or UV degradation products proved even safer than natamycin in toxicology tests, but are inactive biologically.

Natamycin is generally dosed into or onto food as a powder or as an aqueous natamycin suspension. This kind of dosage form leaves the natamycin unprotected under the conditions of processing and use. The natamycin powder, although mixed with excipients such as lactose, may also be sticky to handle and cause dust problems within the food processing plants. Furthermore, natamycin is so highly effective as an antifungal compound that it may adversely affect the processing of the products that it is intended to preserve if this is dependent on desired fungal activity. There is thus a need for a protected dosage form of natamycin.

A general description of natamycin and its current uses may be found in Thomas, L. V. and Delves-Broughton, J. 2003. Natamycin. In: Encyclopedia of Food Sciences and Nutrition. Eds. B. Caballero, L. Trugo and P. Finglas, pp 4109-4115. Elsevier Science Ltd.

Encapsulation technology has been applied to a number of food ingredients, usually to mask flavour or activity. The present invention is based on the realization that unexpected benefits are derivable from the encapsulation of natamycin.

Koontz & Marcey, 2003, J Agric Food Chemistry 51: 7106-7110 describes the formation of a natamycin/cyclodextrin inclusion product. The cyclodextrin acts as host molecules to protect mainly against light, but also low pH, heat and oxidation. However, this natamycin/cyclodextrin complex is not a true encapsulation. A molecule of natamycin will not 'fit' into the cavity of gamma-cyclodextrin, thus it can only be considered a partial encapsulation. Acidic conditions tend to destabilise this kind of complex, releasing the contents of the cyclodextrin molecule and the natamycin molecule is not completely enclosed and protected by the cyclodextrin molecules. Koontz et al. 2003. J Agric Food Chemistry 51: 7111-7114 has also described the stability of natamycin and its cyclodextrin inclusion complexes in aqueous solution.

EP115618 describes an anti-caking antimycotic food ingredient wherein the anti-caking agent is encapsulated and then treated with natamycin to provide antimycotic activity.

US 5,445,949 describes a process for recovery of natamycin by separation of a hydrophobic fermentation product such as natamycin. The process involves a step including encapsulation of a protein but there is no mention of encapsulating the natamycin.

EP-A1-1382261 describes the use of microbial inhibitors such as natamycin for baked bread products, including shelf stable kits for making snacks or meals. The microbial inhibitor is not protected by encapsulation.

Copending patent application US 10/765,210, filed January 28, 2004 relates to the protection of fine bakery goods by spraying the surface of the goods with a natamycin suspension and thus to increase the shelf life of the products.

WO 89/033208 describes a polyene macrolide powder for liposome preparation. The polyene macrolide is encapsulated in liposomes in order to modify the pharmokinetics of the antifungal in systemic diseases. The liposome is intended for pharmaceutical use only.

US 5,821,233 concerns an antifungal composition wherein natamycin is incorporated in porous silica to provide delayed release of natamycin in an aqueous medium.

General descriptions of encapsulation processes may be found in Shahidi, F., and X. – Q., Han. 1993. Encapsulation of food ingredient. Critical Reviews in Food Science and Nutrition 33 (6): 501-547.

The encapsulation of mold inhibitors is described by Ranum, P.,1999. Encapsulated mold inhibitors – the greatest thing since sliced bread in Cereal Foods World, Vol 44, No 5, p. 370 - 371.

US patent 5,204,029 discloses a process for preparing edible microcapsules which contain a multiplicity of liquid cores. In the process, a water-in-oil emulsion, with the active ingredient dissolved in an inner aqueous phase, is spray cooled, which causes the solidification of the fat phase and the entrapment of the aqueous phase as minute droplets dispersed in a microcapsule. This process, however, leads to very unstable microcapsules from which the water phase migrates from the inner part of the microcapsule to an outer part. This further results in the condensation of the water on the wall of a container.

Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Vol. 15, pp. 473 to 474, discloses a process in which liquids are encapsulated using a rotating extrusion head containing concentric nozzles. The process is only suitable for liquids or slurries, and the products of the process are large beads having meltable coatings, such as fats or waxes. However, the microcapsules containing a single liquid droplet as a core are very susceptible to rupture.

In their article "Mass preparation and characterization of alginate microspheres" in Process Biochemistry 35 (2000) 885 to 888 Mofidi, N. et al. describe a method for mass preparation of microspheres, in which method a sterilized alginate solution is prepared and the solution is then poured into a reactor containing a non-aqueous phase,

while being stirred. An emulsion of alginate microdroplets is formed and an appropriate amount of the cross-linker is added. Microspheric alginate-gel particles fell to the bottom and they were collected by filtration.

Similarly, Wong, T.W. et al in J. Microencapsulation, 2002 Vol. 19, no 4, 511 to 522, describe release characteristics from pectin microsphperes and the method for preparing these microspheres. In this method, pectin microspheres are prepared by a water-in-oil emulsion technique, in which minute droplets of pectin containing an active ingredient dispersed in a liquid hydrophobic continuous phase are hardened and collected by filtration.

Microencapsulation by a coacervation-phase separation process is known from an article by Joseph A. Bakan in Controlled Release Technologies, 1980 by Agis F. Kydonieus. The process consists of a series of three steps carried out under continuous agitation: (1) formation of three immiscible chemical phases; (2) deposition of the coating; and (3) rigidization of the coating.

Sanghvi, S.P. and Nairn J.G. have studied the effect of viscosity and interfacial tension on the particle size of cellulose acetate trimellitate microspheres. The results are presented in their article in J. Microencapsulation, 1992, Vol. 9, no 2, 215 to 227.

In their article in Lebensm. -Wiss. u. -Technol., 33, 80 to 88 (2000) Lee, S.J. and Rosenberg, M. describe a double emulsification and heat gelation process for preparing whey protein-based microcapsules. The microcapsules prepared according to the described process are whey protein-based microcapsules containing an apolar core material.

In their article in Science Vol. 298, 1 November 2002, Dinsmore et al. describe selectively permeable capsules composed of colloidal particles. The capsules are fabricated by the self-assembly of colloidal particles onto the interface of emulsion droplets. After the particles are locked together to form elastic shells, the emulsion

droplets are transferred to a fresh continuous-phase fluid that is the same as that inside the droplets.

The documents mentioned in this specification should be considered incorporated herein by reference.

A problem associated with the prior art natamycin dosage forms is that they leave the natamycin unprotected. The efficacy and application of natamycin is compromised by the lability of the preservative to conditions of heat, high and low pH, light and oxidation. There is a need to protect the natamycin and also to provide release of the active natamycin in a controlled manner from the dosage form.

The present invention seeks to overcome the problems of the known natamycin dosage forms, as described above, by providing capsules which are stable against processing conditions and which provide a controlled and/or sustained release of the natamycin.

Brief description of the invention

The present invention is based on the use of encapsulation to protect the natamycin molecule from degradation during adverse conditions and/or to protect process ingredients from exposure to natamycin during processing. The present invention comprises the encapsulation of natamycin by various processes in order to protect it from such degradation or in order to protect the ingredients from such exposure, and the encapsulated natamycin food product itself and its use as a food preservative.

An object of the present invention is thus to provide a natamycin dosage form comprising microcapsules, where natamycin is encapsulated within a physiologically acceptable shell to provide a protected food preservative natamycin product.

An object of the invention is also a process for producing a natamycin dosage form comprising co-processing natamycin with a physiologically acceptable encapsulating material to cause said material to encapsulate said natamycin within a shell, and recovering a protected food preservative natamycin product.

A further object of the invention is a method for the preservation of a food product comprising adding to the food product an effective food-preserving amount of natamycin which is encapsulated within a physiologically acceptable shell.

A further object of the invention is a preserved food product which comprises as a preservative an effective food preserving amount of natamycin which is encapsulated within a shell. The food product is preferably selected from salad dressings, acidic dairy products (including natural cheese, cottage cheese, acidified cheese, cream cheese, yoghurt, sour cream, processed cheese), fruit juices, acidic drinks, alcoholic drinks (including wine and beer), chilled dough and cooked or uncooked bakery products, dairy fillings and toppings for baked goods, surface glazes and coatings for bakery items and other heat-processed items, condiments, dips, purees, pickles, marinades, marinated meat or poultry, breaded meat or poultry, pizza toppings and bases, fast food products, kits for making snacks or meals, kits for making bakery products, pet food, broiler feed and any other acidic, heat-processed and/or fungal fermented food products.

An especially preferred preserved food product is a sliced or cut bakery product, especially sliced bread, wherein encapsulated natamycin has been incorporated into the dough before cooking and provides preservation of the bakery product after baking.

Another preferred preserved food product comprises an acidic food product, into which pH-protected natamycin of the present invention has been incorporated.

The objects of the invention are achieved by the microcapsules, processes, methods and products defined in the independent claims. Preferred embodiments of the invention are disclosed in the dependent claims.

The invention is based on the concept of protecting the active natamycin ingredient by encapsulating it within a physiologically acceptable shell material, for example a

hydrophobic material or a hydrocolloid or any other suitable encapsulating material or a mixture or combination thereof.

The encapsulation of the natamycin is performed by processes which are novel in combination with natamycin and which provide unexpected benefits to the food industry. The encapsulation processes and encapsulating materials or shell materials are selected depending on the nature of the continuous phase in the food application. The shell material must be water-insoluble if the continuous phase of the food application is water-based, and vice-versa in order to provide slow and/or delayed release as well as protection/segregation.

Suitable encapsulating processes comprise fluidized bed processes, liposome encapsulation processes, spray drying processes, spray cooling processes, extrusion processes, co-extrusion processes (such as centrifugal co-extrusion), coacervation processes and combinations thereof.

In a special double encapsulation process, the present invention provides a microcapsule which comprises a solidified hydrophobic shell matrix, an encapsulated aqueous bead or beads encapsulated in the solidified hydrophobic shell matrix, and natamycin as an active ingredient incorporated in the encapsulated aqueous bead or beads.

This natamycin dosage form is provided by a double encapsulation method for preparing microcapsules, which method comprises the steps of

a) providing an aqueous phase and natamycin incorporated in the aqueous phase,

b) providing a hydrophobic phase in melted form,

c) incorporating or dissolving an encapsulating material or mixture of encapsulating materials in the aqueous phase or in the hydrophobic phase,

d) combining the aqueous phase with the hydrophobic phase and homogenizing or mixing the combined phases to form a water-in-oil emulsion,

e) encapsulating the aqueous phase in the emulsion, thus converting the liquid aqueous phase into encapsulated aqueous beads, whereby a dispersion comprising aqueous beads is formed and the natamycin is incorporated in the aqueous beads, and

f) processing the dispersion obtained in step e) to form microcapsules where the encapsulated aqueous beads are further encapsulated in the solidified hydrophobic shell matrix.

The encapsulation process of the present invention may also include gelling, cross-linking, coacervation, sintering or any other suitable means. In the above double encapsulation this results in a dispersion where encapsulated aqueous beads comprising the active natamycin ingredient are dispersed in the hydrophobic phase. The dispersion is cooled below the melting or dropping point of the hydrophobic phase by any suitable process, which results in the formation of microcapsules. The cooling process can be performed, for example by spray cooling or fluidized bed cooling. The microcapsules comprise a number of encapsulated aqueous beads, which further contain the natamycin, and the encapsulated aqueous beads are further encapsulated in a solidified hydrophobic shell matrix.

An advantage of the present invention is that the natamycin is protected by the shell and that the release of the natamycin from the capsules can be controlled. The release rate may be controlled, for instance, by the choice and the amount of the shell material. Thus, the release rate may be controlled by the melting of the hydrophobic shell or by the diffusion of water into the capsule and subsequent migration of natamycin outside the capsule. The release rate of natamycin from the capsules may be selected according to the intended use by selecting a suitable encapsulating material. The release of the natamycin from the capsules of the present invention can be controlled and the release can be initiated in various ways, for example by heat treatment, e.g. by heating, such as in a microwave oven or baking oven, or by freezing, by stress treatment or by any other suitable process. The release of the active ingredients from the capsules of the present invention can also be sustained or it can happen very slowly.

Based on the present disclosure, the person skilled in the art is able to select a suitable encapsulation process as well as the right type and amount of shell material to be used in any one specific food application based on the conditions required to protect and to release the natamycin in accordance with the present invention.

The new improved natamycin dosage form of the present invention enables the use of natamycin in a wide variety of applications, for example in various new applications in the food /feed or pharmaceutical fields.

Yet another advantage of the method of the invention is that it enables a high production capacity to be achieved while the costs are still low.

In the following, the invention will be described in greater detail by means of preferred embodiments and with reference to the examples.

Detailed description of the invention

The present invention relates to a natamycin dosage form comprising natamycin which is encapsulated within a physiologically acceptable shell to provide a protected natamycin product. The preferred dosage form comprises natamycin encapsulated in microcapsules.

The main object of the invention is to improve the use of natamycin in the food industry and, consequently, the shell of the natamycin dosage form of the present invention should be made of a physiologically acceptable material suitable for addition to a food product. The shell provides protection for the natamycin and it should be effective in substantially retaining said natamycin within said shell during processing of food products. Once introduced into a food product, the shell should be effective in providing slow or delayed release of the encapsulated natamycin into the food product.

Most preferably, the natamycin dosage form of the present invention has a shell which is effective in protecting the encapsulated natamycin from degradation by conditions prevailing in the production of a product whereto the encapsulated natamycin is added,

and/or in protecting food ingredients from unwanted attack by natamycin at the wrong time, as well as in providing release of natamycin in said finished product.

The term "food" as used in the present specification and claims refers generally to edible products and beverages of the food and feed industry. The edible products in question are mainly nutritive and/or enjoyable products requiring preservation for their storage between the time of production and eventual use.

The term "physiologically acceptable" likewise refers to materials acceptable and intended for ingestion in connection with food and feed products.

Any suitable food grade coating material may be used as the physiologically acceptable shell material. However, preferred materials are selected from the group consisting of hydrophobic materials, hydrocolloid materials and mixtures or combinations thereof.

The preferred hydrophobic material is chosen from resins and lipids and combinations thereof. The lipids include fatty acids, fats, oils, emulsifiers, fatty alcohols, waxes and mixtures or combinations thereof. The preferred hydrocolloids comprise soluble or dispersible coating materials selected from food grade gums, polysaccharides, proteins, shellac and mixtures or combinations thereof.

Typical hydrocolloid shell materials are selected from cellulosic derivatives (including hydroxy propyl methyl cellulose, carboxy methyl cellulose, methyl cellulose, microcrystalline cellulose and mixtures thereof) with or without stearic acid, zein, shellac and mixtures or combinations thereof.

Generally, the shell on the natamycin dosage form of the invention is provided by co-processing natamycin with an encapsulating material, which is in an aqueous or lipidic solution or suspension or in a molten state.

The natamycin which is to be encapsulated may be in liquid form such as in an aqueous suspension or it may be encapsulated as a dry powder. In a preferred process the shell is provided by co-processing the natamycin with a molten encapsulating material.

A special form of encapsulated natamycin is provided by a doubly encapsulated natamycin dosage form, which comprises microcapsules having a solidified hydrophobic shell matrix, encapsulated aqueous beads which are further encapsulated in the solidified hydrophobic shell matrix, and natamycin incorporated in the encapsulated aqueous beads.

The percentage of active natamycin in the protected natamycin product of the present invention is from 1 to 80% by weight. A preferred amount of natamycin is between 15 and 50% and the most preferred amount is between 30 and 40% by weight.

The process according to the present invention for producing the natamycin dosage form of the invention comprises co-processing natamycin with an encapsulating material to cause said material to encapsulate said natamycin within a shell, and recovering a protected natamycin product.

The encapsulating process is preferably selected from a fluidized bed process, liposome encapsulation, spray drying, spray cooling, extrusion, centrifugal co-extrusion, coacervation and mixtures thereof. Fluidized bed encapsulation and coacervation are the most preferred processes for providing the natamycin dosage form of the present invention.

In a preferred fluidized bed encapsulation natamycin is co-processed with an encapsulating material in an aqueous solution or suspension or in a molten state to provide a shell around the natamycin.

In a preferred coacervation process, an encapsulating material comprising a hydrocolloid or a mixture of hydrocolloids is used to provide the shell.

A special coacervation process of the invention comprises a double encapsulation including the steps of providing an aqueous phase and natamycin incorporated in the aqueous phase, providing a hydrophobic phase in a molten form, incorporating or dissolving an encapsulating material or mixture of encapsulating materials in the aqueous phase or in the hydrophobic phase, combining the aqueous phase with the hydrophobic phase and homogenizing or mixing the combined phases to form a water-in-oil emulsion, encapsulating the aqueous phase in the emulsion, whereby a dispersion comprising encapsulated aqueous beads is formed and the natamycin is encapsulated in the aqueous beads, and processing the dispersion obtained to form microcapsules where the encapsulated aqueous beads are further encapsulated in solidified hydrophobic shell material.

The novel natamycin dosage form of the present invention provides a novel method for the preservation of food products, which comprises adding to a food product an effective food-preserving amount of natamycin which is encapsulated within a shell. The encapsulated natamycin may be added to the food product prior to or in connection with the production of the food product. The shell is effective in protecting the encapsulated natamycin from degradation by conditions used in the production or storage of the food product and/or in protecting the ingredients of the food product from the antifungal effect of the natamycin, and it provides release of natamycin in the food product.

The encapsulation protects the natamycin from degradation by conditions such as heat, light, oxidation and high or low pH.

A special benefit is provided by a preferred embodiment of the invention when the encapsulated natamycin is included in a dough prior to the cooking of a yeast-leavened bakery product since the yeast is protected by the encapsulating shell from direct contact with the natamycin during the leavening.

Furthermore, the encapsulated natamycin is preferably protected against the heat of the baking by the shell. Natamycin is degraded by exposure to heat. During baking, which is typically performed at temperatures ranging from 180 to 300°C, natamycin degradation would significantly reduce the level of active natamycin in the finished baked product. By selecting an encapsulating material having a sufficient heat stability, the heat degradation of natamycin can be substantially reduced. During baking and/or after baking, the shell releases the natamycin so that the finished product is effectively protected against fungal attack.

A preferred use of the present invention comprises use of the novel natamycin dosage form in dough for bread, which is to be sliced for sale. The natamycin released from within the capsule shell in the finished product protects the individual cut bread slices from fungal attack.

Sliced bread is a very convenient food product for consumers. However, the slicing provides an additional process step in the production, and one which is typically performed after the bread has cooled after baking when the product is very susceptible to fungal attack. When the slicing is performed, contamination may take place and as a result, the sliced product will show fungal growth between the slices during storage. The bread slicing exposes a much greater surface area of the bread to contamination particularly by molds.

The copending patent application US 10/765,210, filed January 28, 2004 and included herein by reference, discloses the protection of fine bakery goods by spraying the surface of the goods with natamycin and thus to increase the shelf life of the product. However, it is impossible to apply natamycin between the slices of sliced bread. The present invention provides a solution to the problem of protecting sliced bread by natamycin.

The present invention provides a preserved food product which comprises as a preservative an effective food preserving amount of natamycin which is encapsulated within a shell. Such food products are preferably selected from salad dressings, dips,

salsa sauce, ketchup, purees, pickles, acidic dairy products (including natural cheese, cottage cheese, acidified cheese, cream cheese, yoghurt, sour cream, processed cheese), fruit juices, acidic drinks, alcoholic drinks (including wine), cooked and uncooked bakery products, chilled dough and similar bakery items, dairy fillings and toppings for baked goods, surface glazes and coatings for bakery items and other heat-processed items, marinades, marinated meat or poultry, breaded meat or poultry, fast food products, kits for making snacks or meals, kits for making bakery products, and combinations thereof. The present invention also provides preserved heat processed pet foods and other feed products such as dog or cat food and broiler feed.

In a preferred embodiment of the invention natamycin is protected against pH attack by a process comprising encapsulating natamycin within a shell material to provide an encapsulated natamycin which is protected against degradation caused by low or high pH, the shell material being sufficiently resistant to protect the encapsulated natamycin from degradation by pH and said shell material providing a slow and/or delayed release of natamycin.

Natamycin in solution is fairly stable at neutral pH but is easily degraded, especially at room temperature when the pH rises above pH 10 or sinks below pH 4.5, and especially below pH 4. Thus, for instance natamycin included in acidic products will gradually degrade and will consequently leave the product unprotected at storage and use. The rate of natamycin degradation increases as the temperature is increased.

Many acidic products, such as salad dressings and condiments are stored at ambient temperature and used during a prolonged space of time even after opening of the package. Acidic beverages such as fruit juices can be stored at ambient temperature and may be open to fungal attack. Marinades and marinated meat and poultry are typically stored for a prolonged time at ambient temperature. Many acidic dairy products are stored at ambient or chilled temperatures and may be spoiled by fungal growth. When encapsulated natamycin is added to such products, the encapsulation protects the enclosed natamycin and slowly allows it to diffuse into the product to

replace any degraded natamycin thus keeping the amount of active natamycin at a suitable antifungal level in the product.

The encapsulated natamycin of the present invention provides similar benefits in other acidic products, especially those that are stored at ambient temperature.

The encapsulating shell may also be designed to protect the natamycin against any heat during processing of the acidic food product, such as pasteurization at temperatures of typically 60 to 120°C and more often 60 to 95°C.

The processes used for the encapsulation are briefly outlined below.

Coacervation is a process which works for both water- and fat-based applications since the shell is crosslinked and not soluble in either water or fat.

Fluidized bed coating is suitable for food applications where the continuous phase is water, the possible coating materials include lipids (mono-, di-, triglycerides, fatty acids, waxes and mixtures) applied from the melted form, water-insoluble polymers applied from an ethanolic solution (such as zein and shellac). For applications where the continuous phase is fat, the coating materials include natural, modified or synthetic hydrocolloids (carrageenan, alginate, pectin, locust bean gum (LBG), hydroxypropyl methylcellulose (HPMC), methycellulose) with or without additives (such as film forming agents) applied from a water solution or suspension. The particle size of the natamycin should be over 100 μm, preferably over 150 μm.

A double encapsulation according to the present invention is suitable for fat-based food applications. The inner phase might be composed of water containing a dissolved natamycin/b-cyclodextrin complex and any gelled/crosslinked hydrocolloids or might be composed of glycols (such as ethylene glycol) containing dissolved natamycin and gelled/crosslinked zein.

In a liposome encapsulation natamycin could be incorporated in the lipidic bilayer of the liposome phase.

Spray cooling is a process suitable for water-based food applications. Natamycin is typically incorporated and suspended in melted lipid (mono-, di-, triglycerides, fatty acids, waxes and mixtures) and atomized in cool air to form solid particles containing encapsulated natamycin.

Spray drying is suitable for fat-based food applications. Natamycin is typically incorporated and suspended in aqueous solution of hydrocolloids (gum arabic, modified starch, maltodextrins, whey proteins, caseinate, or the like) with or without additives (such as emulsifiers) and the mixture is atomized in hot air to evaporate the water and form a solid particles containing encapsulated natamycin.

Extrusion is a process which is mainly suitable for fat-based food applications and centrifugal coextrusion is suitable for water-based food applications.

Encapsulation in crosslinked hydrocolloid beads is suitable for both water- and fat-based food applications. A suspension of natamycin (alone or in combination with a suitable solvent) is typically prepared in aqueous alginate, low ester pectin or any other "crosslinkable" hydrocolloids and added dropwise or sprayed into a bath of aqueous calcium ions. The crosslinked beads or particles containing the encapsulated natamycin are collected by filtration and used as is (wet) or dried by fluidized bed or any other suitable means.

Food products, which are especially suitable for being preserved by the novel natamycin dosage form of the present invention include fat-containing acidic products such as salad dressings and acidic dairy products (natural cheese, cottage cheese, acidified cheese, cream cheese, yoghurt, sour cream). Many of these products can be preserved with natamycin in non-encapsulated form and they will generally keep well, if chill stored. However, if they are stored at ambient temperature, degradation of the natamycin is a problem. This problem is solved by the encapsulated natamycin of the present invention.

In USA salad dressings are usually cold-processed, in which case contaminant yeasts and moulds are potential spoilage contaminants. The combination of ambient temperature storage and low pH causes rapid natamycin degradation. If non-encapsulated natamycin, which is added when the dressings are first made fails to rapidly kill all the contaminant yeasts, and if any mould spores are present (these are not normally killed by natamycin), there is potential for fungal growth/spoilage once natamycin levels drop.

By use of the encapsulated natamycin of the present invention the acidic food products may be stored at ambient temperature for up to 12 months.

The preferred processes for encapsulation for the acidic food products comprise coacervation and fluidized bed encapsulation.

The coacervation process typically involves 1) preparing a suspension of natamycin in an aqueous solution of hydrolloids, 2) decreasing the solubility of the hydrocolloids, to cause a phase separation and the formation of a hydrocolloid-rich third phase by use of additives or by adjusting the temperature, 3) processing the tri-phasic system in such as way as to deposit the newly formed coacervate phase onto the suspended natamycin particles and finally 4) hardening the hydrocolloid shell around the natamycin by adjusting the temperature, adding chemical or enzymatic crosslinker or otherwise followed by the isolation of the microcapsules by freeze-drying, spray-drying, filtration or any other means.

In the fluidized bed encapsulation the appropriate shell material is typically applied from aqueous solutions or suspensions include HPMC, methylcellulose, microcrystalline cellulose and other cellulosic derivatives with or without stearic acid, other fatty acids, or other hydrophobic additives. Appropriate shell material applied from the molten state include lipids, mono-, di- or tro-glycerides, fatty acids, fatty alcohols, waxes, or mixture thereof or any other meltable hydrophobic material.

Another type of food product which derives great benefits from the present invention is fruit juice and acidic drinks. Benefits are also derived for processed fruit, low pH sauces, such as ketchups and purees, salsa sauces, condiments, dips, pickles, etc, alcoholic drinks such as wine or beer and the like. These liquid products may contain fat (acidified fruit milk drinks, etc). They may be pasteurised. The combination of pasteurisation at low pH, but more importantly acid pH and ambient temperature storage results in degradation of non-protected natamycin. If post-processing contamination with yeasts or moulds has occurred, or heat-resistant mould spores (e.g. *Byssochlamys, Talaryomyces*) or yeast ascospores survive the processing, fungal growth will occur once natamycin levels are degraded.

Animal feed products such as dog and cat food or broiler feed is often heat processed during the production thereof and then stored at ambient temperature. The encapsulated heat-stable natamycin of the present invention can conveniently be used to protect such feed products.

In liquid products such as juices or wines, the shell material should be made of a material which does not disturb the clarity of the liquid.

When the natamycin is added in the form of the novel capsules of the present invention, the shell will slowly dose out small amounts of natamycin and keep the liquid products free of fungal growth for extended periods (3-9 months) of storage at ambient temperature. The encapsulation provides a special benefit for heat-treated acidic liquids since the shell protects the natamycin both from heat and acid attack.

Natamycin is a preservative which may also be used to advantage in bakery products. Most baked goods are susceptible to mould spoilage due to aerial contamination with mould spores after baking. Propionate is commonly incorporated into bread and other yeast-leavened doughs as an anti-mould agent. The anti-yeast activity of propionate is much weaker compared to its anti-mould activity in these doughs. Although propionate has a slight inhibitory effect against the bakers' yeast, this is acceptable.

Natamycin cannot be used in this way because it is strongly active against both yeasts and moulds. Encapsulation of the natamycin prevents the natamycin activity against the bakers' yeast until after the leavening is complete. It also protects the natamycin during the baking process. This is particularly useful for products such as sliced breads that have a large surface area exposed to air contamination.

The encapsulation processes of the present invention are described in some detail below:

1. Fluidized bed encapsulation

The natamycin is preferably used in dry powder form. If the raw natamycin particle size is too fine, the powder can be agglomerated in an suitable equipment using a binder solution (solution of sticky hydrocolloids such as alginate or maltodextrine) in order to obtain a dense powder of particle size between 100-500μ. The appropriate powder is then introduced into the coating chamber of a fluidized-bed microencapsulation unit and fluidized at inlet air flow rate of 20-135cm/s at the bottom plate and temperature between 5 to 75 °C are used to fluidized the particles. A coating material is then sprayed onto the fluidized bed of antimicrobial using a double fluid nozzle and high pressure atomization air.

In one example, a melted mixture of triglyceride and additives is sprayed onto the antimicrobial powder to form a continuous layer of fat around each individual particle as the melted fat spread and solidifies on the particles. The amount of fat applied can be up to 60%, but no usually no lower than 15% w/w.

In another example, a dispersion or solution of coating material in water and/or ethanol is sprayed onto the fluidized particles and the fluidization air is used to evaporate the solvent or the water, which leaves behind a continuous film of coating material on the antimicrobial particles.

Examples of coating material in this case include any hydrocolloids (polysaccharides, proteins, shellac, zein or any other soluble or dispersible coating materials).

2. Liposome encapsulation.

Typically, liposomes are prepared using a dehydration-hydration method involving organisc solvent, such as the one described below. However, solvent-free methods, more suitable for food ingredients, are also available using microfluidization or homogenization devices or by repeated freeze-thaw cycles.

A typical procedure for the preparation of liposome-encapsulated natamycin involves the preparation of a solution of 1 g of a bilayer-forming lipid and 100 mg of cholesterol or alpha-tocopherol in a suitable organic solvent and evaporating the solvent so as to form a thin dry lipid film on the bottom of the container.

After thorough drying of the lipid film, 1L of water containing natamycin at or over the saturation concentration (natamycin solubility can be increased if desired by the formation of alkaline salts) is added to the container and the mixture is thoroughly mixed or homogenized.

The resulting suspension of multilamellar vesicle (MLV) can be further processed by microfluidization and or sonication to form smaller more homogenous small unilamellar vesicle (SUV). The suspension of liposome-encapsulated natamycin can be added directly to the application or dried by lyophilization or any other appropriate drying procedures.

3. Spray drying

Natamycin can be encapsulated in a matrix of hydrocolloids by means of spray drying. In a typical procedure, an aqueous natamycin suspension in which a hydrocolloid or a mixture of hydrocolloids is dissolved (water-soluble polysaccharides, proteins, modified polysaccharides with or without film forming agents such as oligosaccharides, plasticizers, emulsifiers or other additives) is prepared at near-neutral pH (to minimize degradation of natamycin). Then, the mixture is pumped through an atomizer (rotary atomizer, pressure nozzle, double fluid nozzle or any other atomization device) into a drying chamber co- or counter-currently with heated air.

The temperature of the heated air is typically between 160 and 200 °C, can be as high as 300 °C, but is preferably in the range of 100-160 °C. Evaporation of water yields a free flowing powder of microcapsules containing dispersed natamycin in the dry hydrocolloid(s) matrix.

4. Spray cooling

In spray cooling/chilling/congealing of natamycin, the powdered natamycin is dispersed in a molten lipid or mixture of lipids (mono-, di-, tri-glycerides, esterified glycerides, animal, vegetable or mineral waxes, any other meltable material at temperature between 45 and 125 °C) with or without the aid of surface-active additives. The dispersion is then pumped through an atomizer (rotary atomizer, pressure nozzle, double fluid nozzle, spinning disk or any other atomization device) into a cooling chamber co- or counter-currently with cooled air.

The temperature of the cooled air is typically between –10 and 30 °C, but can be as low as –40 °C. solidification of the lipid yields a free flowing powder of microcapsules containing dispersed natamycin in the crystallized lipidic matrix.

5. Extrusion

Encapsulation of natamycin by extrusion can be achieved by processing the powdered natamycin (preferably of small particle size) together with a melted or plasticized polymeric shell material in a double- or single-screw extruder under pressure, followed by the cooling or the drying of the mass coming out of the extruder die and milling or crimpling to the appropriate particle size. The polymeric mass is melted in the extruder at relatively high temperatures in the presence of small amount of water, which causes the mass to become flowable. The mass, in which the natamycin is incorporated, is extruded ánd cooling results in the transformation of the mass into a glassy state which is highly impermeable to oxygen and other hydrobobic external agents. Shell materials suitable for extrusion of natamycin include oligosaccharides, polysaccharides, modified polysaccharide, proteins or mixtures thereof with or without the use of plasticizing, emulsifying or stabilizing additives.

6. Centrifugal co-extrusion

Encapsulation of natamycin by centrifugal co-extrusion is a variation of the spray cooling process. In centrifugal coextrusion of natamycin, the powdered antimicrobial is first dispersed in a molten lipid or mixture of lipids (mono-, di-, tri-glycerides, esterified glycerides, animal, vegetable or mineral waxes, any other meltable material at temperature between 45 and 125 °C) with or without the aid of surface-active additives. The dispersion is then pumped through the inner part of a double fluid nozzle while another stream of molten lipid or mixture of lipids (same as above) is pumped through the outer part of the double fluid nozzle. The nozzle is rotated around its axis so as to break up the stream of melted fat in discrete globules, which are solidified by cooled air. The resulting microcapsules are composed of an outer layer of solidified fat encapsulating a core of solidified fat containing dispersed natamycin.

7. Coacervation

The natamycin dosage form of the present invention can be formed by coacervation. The coacervation of the shell material, such as hydrocolloid, is carried out by using any suitable coacervation process. The coacervation can be performed for example by adding salt(s), sugar(s), or other additives, which cause the phase separation of the hydrocolloid(s). The coacervation can also be performed by subjecting the emulsion to heating, cooling, pH change by adding acid or base, which cause the phase separation of the hydrocolloid(s). The deposition of the coacervated phase around the aqueous phase is spontaneous and driven by surface tension forces. The coacervate layer can afterwards be subjected to cross-linking or hardening by any suitable means, which are known to persons skilled in coacervation.

The shell materials suitable for coacervation are selected form the group comprising any mixture of one or many ionic hydrocolloids and one or many amphoteric hjydrocolloids, such as any mixture of polysaccharides and proteins, gelatine/arabic gum, gelatine/CMC, any proteins/ionic hydrocolloids, any combination of hydrocolloids and a solubility-reducing agent such as salts, sugars, acids or bases.

8. Double encapsulation

According to a special aspect of the present invention, the natamycin suspension is double encapsulated in microcapsules. In that case, the natamycin is first incorporated (suspended) in an aqueous phase containing encapsulating material, such as hydrocolloid or any other suitable encapsulating material or mixture thereof, and the aqueous phase is encapsulated, for example by gelling, cross-linking, coacervation, sintering or by any other suitable means, and the resulting encapsulated aqueous bead or beads is/are further encapsulated in a solidified hydrophobic shell material.

A hydrophobic shell material is selected based on desired properties of the capsules, for example based on the intended use of the capsules, storage temperature, etc. The hydrophobic shell material should have a melting point above 45 °C so that it can be stored at room temperature, in general any hydrophobic material can be used if the capsules are stored below the melting temperature of the hydrophobic material.

In this application, melted form means that the hydrophobic phase is at the lowest temperature at which the hydrophobic phase is sufficiently fluid to drip, as determined by test method ASTM D 566 or D 265.

The hydrophobic shell material useful in the various processes of the invention is selected from the group comprising fats, oils, waxes, resins, emulsifiers or mixtures thereof, which are preferably food-grade. Preferably the hydrophobic shell material is selected from the group comprising animal oils and fats, fully hydrogenated vegetable or animal oils, partially hydrogenated vegetable or animal oils, unsaturated, hydrogenated or fully hydrogenated fatty acids, unsaturated, partially hydrogenated or fully hydrogenated fatty acid monoglycerides and diglycerides, unsaturated, partially hydrogenated or fully hydrogenated esterified fatty acids of monoglycerides or diglycerides, unsaturated, partially hydrogenated or fully hydrogenated free fatty acids, other emulsifiers, animal waxes, vegetable waxes, mineral waxes, synthetic waxes, natural and synthetic resins and mixtures thereof.

Animal oils and fats are such as, but not restricted to, beef tallow, mutton tallow, lamb tallow, lard or pork fat, sperm oil. Hydrogenated or partially hydrogenated vegetable oils are such as, but not restricted to, canola oil, cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, linseed oil, tung oil and castor oil. Free fatty acids are such as, but not restricted to, stearic acid, palmitic acid and oleic acid. Other emulsifiers are such as, but not restricted to, polyglycerol esters, sorbitan esters of fatty acids. Animal waxes are such as, but not restricted to, beeswax, lanolin, shell wax or Chinese insect wax. Vegetable waxes are such as, but not restricted to, carnauba, candelilla, bayberry or sugarcane waxes. Mineral waxes are such as, but not restricted to, paraffin, microcrysalline petroleum, ozocerite, ceresin or montan. Synthetic waxes are such as, but not restricted to, low molecular weight polyolefin, polyol ether-esters and Fisher-Tropsch process synthetic waxes. Natural resins are such as rosin, balsam, shellac and zein.

The hydrocolloid shell material of the invention is any food-grade hydrocolloid which is susceptible to encapsulation by the processes of the invention.

The material is selected from the group comprising hydrocolloids, sodium alginate, gum arabic, gellan gum, starch, modified starch, guar gum, agar gum, pectin, amidified pectin, carrageenan, xanthan, gelatine, chitosan, mesquite gum, hyaluronic acid, cellulose derivatives such as cellulose acetate phtalate, hydroxy propyl methylcellulose (HPMC), methyl cellulose, ethyl cellulose and carboxy methyl cellulose (CMC), methyl acrylic copolymers, such as Eudragit®, psyllium, tamarind, xanthan, locust bean gum, whey protein, soy protein, sodium caseinate, any food-grade protein, shellac, zein, any synthetic or natural water-soluble polymers, and mixtures thereof.

According to a special double encapsulation embodiment of the present invention, the microcapsule comprises a solidified hydrophobic shell matrix, a gelled or cross-linked aqueous hydrocolloid bead or beads encapsulated in the solidified hydrophobic shell matrix, and natamycin suspended in the gelled or cross-linked aqueous hydrocolloid bead or beads.

The gelled hydrocolloids have a gelling temperature above room temperature. Examples of gelled hydrocolloids include carrageenan, gelatine, guar gum, agar gum, starch, modified starch and mixture of xanthan and locust bean gum, mixture of carrageenan and locust bean gum and mixture of any gelling hydrocolloids and other non-gelling hydrocolloids.

The cross-linking of the hydrocolloids is carried out by using cross-linking agents or by a variety of mechanisms. If the hydrocolloid is a protein or polysaccharide bearing amino groups, it can be cross-linked by using dialdehydes, such as glutaraldehyde. If the hydrocolloid is a polysaccharide, such as sodium alginate, gellan gum or pectin, it can be cross-linked with multivalent ions, such as calcium or magnesium. The cross-linking can also be carried out by other mechanisms, such as heating, pH adjustment, applying pressure or by enzymatic cross-linking. Proteins, for example, can be cross-linked by subjecting a protein to a high pressure, preferably from 5 to 200 bar, and/or by subjecting a protein to a temperature which is above the denaturation temperature of the protein. The enzymatic cross-linking of proteins can be carried out for example with transglutamidase. Based on the hydrocolloid used, a person skilled in the art is able to decide which method of gelling or cross-linking is used.

The capsules of the present invention are preferably microcapsules and comprise 1 to 80 %, preferably 15 to 50%, most preferably 30 to 40% natamycin encapsulated in the hydrophobic or hydrocolloid shell. The size of a microcapsule is approximately between 40 to 800 microns, preferably 100 to 150 microns. The amount of natamycin encapsulated within the shell of a microcapsule may vary, depending on the intended use of the microcapsules. The size of the microcapsules of the present invention may also vary depending on the intended use.

The aqueous phase mentioned in the present specification means water or a mixture of water and any other water-miscible solvents, such as ethanol, ethylene glycol or glycerol. The aqueous phase may also contain additives, such as carbohydrates, such as monosaccharides or oligosaccharides to modify the properties of the hydrocolloid gel, inorganic salts to modify the properties of protein gels, preservatives to avoid

deterioration of the microcapsules by bacteria or fungus or emulsifiers as processing aids, sorbitan tristearate or other emulsifiers as crystal form modifier, hydrophobic natural or synthetic polymers to modify mechanical properties of the capsule, plastizisers, preservatives to avoid deterioration of the capsules.

The encapsulated natamycin described above can be used in a wide variety of applications in food industry and in pharmaceutical applications.

The capsules of the present invention can be used in a great variety of applications, depending for example on the properties of the capsules, the hydrocolloid, the hydrophobic material or the size of the capsules. A controlled release of the active ingredients from the capsules can be achieved by the present invention. The release of the active ingredients from the capsules can be controlled by initiating the release in various ways, for example by heat treatment, by heating in a microwave oven or baking oven, by pH, by light, or by any other suitable process. The release of the active ingredients from the capsules of the present invention can also happen very slowly. The release of the natamycin may also take place upon freezing of the capsules. Freezing causes any water phase inside the capsule to expand, which causes the external shell material to crack. Upon thawing, the natamycin is quickly released from the microcapsule.

In bakery, for example, delayed release of natamycin can be achieved with the capsules of the present invention. This is very important in order to avoid inhibition of the required activity of the baker's yeast. Increased heat stability of the natamycin is achieved for example in pasteurised or heat-processed foods. Delayed release of natamycin is also very important for other yeast fermented foods.

The present invention relates to the use of encapsulated natamycin as preservative agent providing slow, controlled and/or sustained release of the natamycin.

Controlled release of natamycin in food products, such as baked goods, pizza, is achieved with the capsules of the present invention. The encapsulated natamycin is

retained in the product until heat, pH, light and/or stress treatment is applied to release the natamycin. Heat can be provided for example by a micro-wave-oven, conventional oven or hot water. Stress can be provided for example by processing conditions or mastication.

Slow release of natamycin for example in processed meat products or in beverages, such as orange juice, is achieved with the encapsulated natamycin of the present invention. The natamycin preservative agent in the capsules of the present invention is slowly released in the product as it is naturally degraded. This effectively prevents growth of fungi or other undesirable micro organisms for a longer period of time than non-encapsulated natamycin, thus ensuring a longer shelf life for the food product. The shell can also provide thermal stability to natamycin so as to survive heat treatment and harsh processing conditions, but to remain active during storage of the processed product.

Delayed release of natamycin in bakery applications is achieved by the capsules of the present invention. Natamycin is useful for extending the shelf life of breads and other bakery products, but at the expense of detrimentally affecting the effectiveness of the yeast. The delayed release allows a more efficient use of the yeast, while also providing the preservative properties after the natamycin is released during baking.

The encapsulated natamycin is also useful in many ready-to-use food products, in snacks and in kits for producing snacks and meals. The encapsulated natamycin ensures the slow and continuous release of natamycin into the product so as to keep the level of active (non-degraded) natamycin high enough to prevent spoilage of the product.

The following examples serve to illustrate the invention

Examples

Example 1

Production of encapsulated natamycin by a coacervation process

First, a solution of gelatine (219 g, isoelectric point = 8) in 6 liters of water at 50 °C was prepared. Secondly, a solution of 219 g of gum acacia was dissolved in 6L of water at 50 °C. The two solutions were mixed together and kept at 45 °C under vigorous stirring. 700 g of Natamax™ SF (Danisco) was added to the aqueous solutions and the pH was rapidly lowered to 4.0 using 1M HCl, after which the temperature was lowered to 5 °C at the rate of approximately 1 °C/min, maintaining the stirring throughout. 36 ml of an 1:1 aqueous solution of glutaraldehyde was added, the pH was re-adjusted to 8.5 using aqueous 1M NaOH and then the temperature was increased back to 45 °C at a rate of approx 2 °C/min. Finally, the whole mixture was spray dried in a spray tower using a double-fluid nozzle mounted in the fountain configuration, air inlet temperature of 180 °C and a spray rate to maintain the outlet air temperature of about 100 °C.

In an alternatively process, 1kg each of gum arabic and maltodextrin (DE 12) are dissolved in the aqueous mixture just prior to spray drying.

Example 2

Fluid bed encapsulation of natamycin

Preprocessing.

If the natamycin particle size is too fine (below 100 micrometers average), the powder is agglomerated to a larger average particle size for easier processing by fluidized bed. Larger average particle size not only makes the process easier, but also allow the use of less coating material while achieving the same protection as with more shell material. Natamycin is agglomerated in an suitable equipment such as a high shear mixer (such as a Lödige mixer using a binder solution (solution of sticky hydrocolloids such as alginate or maltodextrine) in order to obtain a dense powder of particle size above 150, preferably between 200-350 μm and bulk density above 0.4, preferably above 0.7 g/cm^3.

Hotmelt fluid bed encapsulation

3 kg of agglomerated natamycin is introduced into the coating chamber of a Aeromatic-Fielder MP1 fluidized-bed microencapsulation unit and fluidized using inlet air flow rate of 80 cm/s and temperature of 43 °C. A melted hydrogenated triglyceride kept at 85 °C is then sprayed onto the fluidized bed of antimicrobial using a peristaltic pump and a double fluid nozzle set a 2 bar and 2 m^3 of air/h. The fat is applied at around 1 kg/h, in such a way to form a continuous layer of fat around each individual particles as the melted fat spread and solidifies on the particles. Enough fat is applied to reach a final product containing 30 % fat and 70 % natamycin.

Example 3

Extrusion encapsulation of natamycin

A mixture of 60 parts of corn starch, 25 parts of natamycin and 10 parts of polyethyleneglycol and 5 parts of water is mixed together and introduced in a clextral double-screw extruder, the first barrel heated to 40 °C. The mass is treated at 100 °C for just a few seconds in barrels 2 and 3 then cooled down to 45 °C in barrels up to the die. Alternatively, a vacuum pump is installed on the last barrel so as to get rid of the water. The exiting rope is cut into pieces between 250 and 500 μm.

Example 4

Use of encapsulated natamycin in orange juice

Natamycin was encapsulated by a coacervation method as described in Example 1, using either gelatine and acacia as a shell material (NAP03015), or gelatine, acacia and maltodextrin (NAP03023).

The samples, together with natamycin as Natamax™ (Danisco) were added to orange juice (pH 3.85) and heated at 100 °C for 10 minutes. The residual natamycin levels in the juice before and after treatment were tested by HPLC. Samples were diluted in methanol for this assay.

The results are shown in Table 1.

The experiment shows that the microcapsule prevented release of natamycin, so that not all the estimated natamycin present could be detected before the heating step. After heating, the encapsulated natamycin showed recovery levels higher than with the unprotected natamycin.

Table 1. Heat protection of encapsulated natamycin in orange juice

Sample	Theoretical payload based on pure natamycin W/w	Addition level	Actual natamycin added (based on estimated payload)	Detectable natamycin in juice before heating /ppm (% of natamycin added)	Detectable natamycin in juice after heating /ppm (% of natamycin added)
Natamax™	50%	40 ppm	20 ppm	19.7 (98.5%)	5.2 (26%)
NAP03015	80%	80 ppm	64 ppm	33.0 (52%)	21.1 (33%)
NAP03023	36%	140 ppm	50 ppm	14.1 (28%)	14.8 (30%)

Example 5

Use of encapsulated natamycin in vinaigrette

A vinaigrette dressing was prepared containing water (494.6 ml), 10 % vinegar (220 ml), sugar (90 g) and salt (10 g), pH 2.6. Additions of encapsulated and unencapsulated natamycin were made as shown in Table 2. Sample NAP03015 was encapsulated by coacervation as described in Example 1. Sample NAP03007 was encapsulated by spray-cooling with a shell material of triglyceride.

Table 2

Sample	Theoretical payload of pure natamycin	Addition level	Actual natamycin added (based on estimated payload)
Natamax™	50%	40 ppm	20 ppm
NAP03007	40%	100 ppm	40 ppm
NAP03015	80%	50 ppm	40 ppm

The vinaigrette was incubated at 25 °C, and samples assayed for residual natamycin content at regular intervals. The vinaigrette was shaken before each sampling, and a

sample taken for HPLC analysis, which was diluted 1:1 in methanol. The natamycin levels found in the mixed vinaigrette and in the water layer only are shown in Table 3 and 4. The results show that encapsulation protects the natamycin from acid degradation in the vinaigrette, allowing a slow release of the preservative with time. Sample NAP03007 contained only a small amount of unencapsulated natamycin at the beginning of the experiment.

Table 3. Detectable natamycin in a vinaigrette dressing at 25 °C (Sample taken from homogenised dressing)

Days at 25 °C	Natamycin percentage of estimated addition level (based on estimated addition level)		
	Natamax	NAP03007	NAP03015
0	70.5%	1.8%	70%
1	38%	4.5%	50.5%
6	22.5%	19.3%	23.8%
9	13%	29.5%	36.5%
14	10%	40.8%	29%
21	4.5%	17.5%	10.2%

Table 4. Detectable natamycin from the water phase of a vinaigrette dressing at 25 °C

Days at 25 °C	Natamycin % of estimated addition level. (based on estimated addition level)		
	Natamax	NAP03007	NAP03015
0	48%	1.5%	13%
1	25%	2.25%	15.3%
6	8%	2.75%	7.8%
9	8%	13%	5%
14	6%	13.5%	5.3%
21	2.5%	11.8%	4.3%

Example 6.

Use of encapsulated natamycin in bread

A bread is made by preparing a dough containing flour, water, yeast, salt and a dough conditioner. Included in the dough mix is either natamycin or encapsulated natamycin or neither. Both natamycin preparations are added at a potency dosage of 12 ppm

(0.0012 %) on flour weight and these are added together with the other dry ingredients. All ingredients are mixed together thoroughly for between 3 and 10 minutes.

The dough is then given a short resting period after mixing (approx. 5 to 10 minutes) followed by scaling at the required weight. A second rest period is then applied following a second moulding in shape the dough as desired. The dough is then placed into a tin or tray. A leavening period for about 50 minutes at 85 % relative humidity at 40 °C then follows.

The fully proved dough is then baked at between 190 and 230 °C for approximately 15 to 30 minutes.

Bread containing unencapsulated natamycin shows poor leavening, whereas leavening of the encapsulated natamycin proceeds in a similar fashion to the control bread not containing any natamycin. This demonstrates the benefit of encapsulation, which prevents the natamycin from inhibiting the yeast fermentation reaction.

When the bread is cool, the natamycin content in the bread is assayed. The natamycin content from bread containing encapsulated natamycin is higher than that in the bread containing unencapsulated natamycin, indicating the heat protective benefit of encapsulated natamycin. The bread is then sliced and observed over the normal shelf life period for growth of moulds. Delay of mould spoilage is observed for bread containing natamycin. This extension of shelf life is greater for bread containing encapsulated natamycin, which is a reflection of the higher natamycin levels surviving the baking process.

Example 7

Encapsulation of natamycin in a double shell

First, a solution of 15 g kappa-carrageenan in 1000 ml of phosphate buffer at pH 7.0 is prepared at 85 °C. To this is added 300 g of commercial natamycin (Natamax™ SF, Danisco). The resulting mixture is thoroughly mixed. At the same time, a mixture of

1333 g of a vegetable triglyceride (GRINDSTED ® PS 101, m.p. 58°C) and 73 g of acetylated emulsifier (Acetem 50 00) is melted at 85 °C in a water bath. The melted fat mixture is kept under homogenization (Silverson mixer, 8000 rpm) as the aqueous mixture is slowly incorporated. The homogenization is maintained for 5 minutes after the whole aqueous mixture is added and then a solution of 3 g of polysorbate 80 in 40 ml of water is added under constant mixing. The resulting low-viscosity water-in-oil emulsion is then immediately spray cooled in a Niro spray tower using the following parameters: inlet air temperature 10 °C, outlet air temperature 28 °C, rotating atomization wheel speed 10 000 rpm. A free flowing powder is obtained. The incorporation of encapsulated natamycin in an orange juice results in a much more stable natamycin formulation compared to when unencapsulated natamycin is used in the liquid, thus dramatically improving survival rate of the natamycinin the beverage. The encapsulated natamycin, as presented in this example, is released at a rate of only 7 % after three days.

It will be obvious to a person skilled in the art that as technology advances, the inventive concept can be implemented in various ways. The invention and its embodiments are not limited to the examples described above but may vary within the scope of the claims.